REMARKS

The Claimed Invention

The claimed invention is directed to antigen-presenting vesicles free from their natural surroundings comprising a membrane and a major histocompatibility complex (MHC) class I protein.

The Pending Claims

Claims 2-4, 6 and 13 are pending. Claims 2-4, 6 and 13 are directed to an antigen-presenting vesicle.

The Advisory Action

The Examiner did not enter the amendment submitted on the basis that newly added Claim 14 raises new issues which require further consideration and search.

Claims 2-4, 6 and 13 remain rejected under 35 U.S.C. 112, first paragraph, enablement.

Amendments:

Applicants have canceled Claims 2 and 10, and amended Claims 9 and 11-13.

Claims 9, 11 and 12 were amended to become product by process claims. These claims now therefore fall within the elected subject matter for this application. Support is found in originally filed Claims 9, 11 and 12, and on page 2, line 25 through page 3, line 7; page 8, line 23 through page 9, line 8; and page 11, line 36 through page 12, line 14 of the original specification.

New issues

In accordance with the Examiner's suggestion made in the Office action mailed January 16, 2001, Claim 13 was amended to recite "an antigen presenting cell". Support is found on page 5, line 35 through page 6, line 1 of the originally filed specification.

Applicants have made the indicated amendments to place this application in form for allowance or in better form for appeal. The amendments were made earlier but were not

entered by the Examiner on the basis that previously added new Claim 14 would raise new issues requiring a new search. Claim 14 is not included in the above amendments. No new matter has been added by any of these amendments and the Examiner is respectfully requested to enter them.

The Examiner's specific objections and rejections are reiterated below as small indented bold print, followed by Applicant's response in normal print.

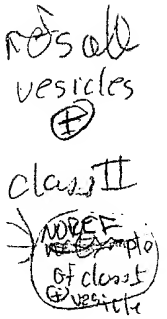
35 U.S.C. 112, first paragraph, enablement

Though applicant's arguments and amendments in their after-final amendment mailed 10-27-00 (Paper No. 15), have been carefully considered, the 112 first rejection is maintained essentially for the reasons of record . Applicants traverse the rejection on the grounds that the specification teaches the skilled artisan how to make and use an antigen presenting vesicle comprising an MHC Class I protein, and specifically, that the instant specification provides guidance on how to isolate exosomes from antigen presenting cells and demonstrates how to use them to stimulate T cells. However, the examiner notes that said guidance regarding the making and using of said exosomes is disclosed in the context of class II only, not in the context of Class I. As repeated from the previous final rejection mailed 1-17-01, the examiner also agrees that the instant specification have described methods of differential centrifugation and isolation of subcellular fractions over sucrose gradients, but the examiner notes that the examples of an antigen presenting vesicle in the instant specification asserted by applicant all refer to the isolation of MIIC and exosomes. In view of the virtual absence of guidance from the instant specification of which vesicles contain class I proteins, and in view of the lack of sufficient guidance in the instant specification regarding how to make and use said vesicles comprising class I proteins, and in view of the lack of predictability concerning MHC Class I expression on exosomes as demonstrated by the post-filing date Zitvogel reference, and in view of the absence of a working definition of "vesicle" in the instant specification, the examiner maintains it would require an undue amount of experimentation on the part of one skilled in the art to make and use the claimed antigen presenting vesicle comprising a MHC Class I protein for the asserted utilities.

1. The term "vesicle" is an art-recognized term

With regard to the Examiner's "absence of a working definition of 'vesicle' in the instant specification", Applicants respectfully point out the term "vesicle" is an art-recognized term which refers to a closed membrane shell derived from cell membranes either by a physiological process, such as by budding, or mechanically, such as by sonication. In the present specification, the term is characterized on pages 1-2, and particularly on page 1, line 24-29 and page 2, lines 1 to 7. The scientific literature is replete with references to vesicles, methods for producing them, and how they may be used. For example, West et al (ref. no. 8;

(1994)) in an article dedicated to transport and maturation of class II MHC and specialized sited involved in peptide acquisition, describe membrane vesicles carrying newly assembled class II MHC complexes. Kleijmeer et al (ref. no. 7; (1994)) studied intracellular distribution of MHC-II molecules and I-chain) in Langerhans cells and teaches MHC-II-enriched compartments with internal membrane vesicles. Riberdy et al. (ref. no. 6; (1994)) describes how class II-invariant chain complexes accumulate intracellularly in large acidic vesicles which contain lysosomal markers. These references are cited on page 1 of the application as filed, in which it is stated "We and others have shown that most of the intracellular MHC class II molecules reside in a lysosome like, MHC-class II-enriched compartment (MIIC) which contains characteristic membrane vesicles and concentrically arranged membrane sheets". The specification of the present application continues by referring to a publication by Amigorena (ref. no. 11; (1994)), in which a "population of class II-enriched vesicles has been discovered in B lymphocytes that accumulate internalized antigen but are distinct from endosomes and lysosomes". On page 2, lines 6-7, the present application refers to secreted vesicles, termed exosomes, and references two articles (ref. no. 13; Harding et al. (1984) and Pan et al. (1985) that describe how vesicles are secreted by reticulocytes and B cells (ref. nos. 5 and 6; Peters (1991) and Riberdy (1994), respectively). In a search of the scientific literature form 1985 to 1995, Applicants have found 8 articles for "vesicles" and "antigen-presenting", 19 articles comprising "vesicles" and "reticulocytes", 24 articles referring to "vesicles" and "B cells", 47 articles describing "vesicles" and "macrophages", 109 articles referencing "vesicles" and "dendritic", and 388 articles discussing "vesicles" and "antigens". These references confirm that the term "vesicle" used in the context of the present application was an art-recognized term at the time the present application was filed, and that methods for studying, making and using vesicles from antigen-presenting cells had been well known at that time.



2. Methods of making vesicles comprising MHC class I protein are provided

Applicants respectfully disagree with the Examiner's statement that "the making... of said exosomes is disclosed in the context of class II only, not in the context of class I". In lines 2-3 of the Abstract of the originally filed specification, it is stated that "Exosomes are vesicles

derived from MHC class II enriched compartments in antigen presenting cells. The exosomes possess MHC II and/or MHC I molecules at their surface and possibly peptides derived from processed antigens in said MHC's." Details are presented in the specification in terms of MHC class II but the specification indicates that vesicles with either one or both of MHC class I and MHC class II are produced by the described methods from appropriate cells (page 6, lines 3-6); "These vesicles preferably will contain major histocompatibility complex (MHC) I and/or II..."(page 6, lines 3-4). Other methods of producing vesicles are also described, such as synthetically-prepared liposomes (page 6, lines 25-29) and by recombinant means (page 6, lines 29-32). Support for making antigen-presenting vesicles is found in page 2, line 25 through page 3, line 7 and page 8, line 23 through page 9, line 8 of the original specification. In each instance, both MHC class I and class II are referenced.

Although the claimed antigen-presenting vesicles are exemplified by identifying the presence of MHC class II molecules, the specification teaches the presence of MHC class I molecules on multivesicular MIICs. The post-filing publication of Zitvogel, *et al.* confirms the operability of the claimed antigen-presenting vesicles, and that multivesicular MIICs can comprise MHC I and/or MHC I molecules. The Zitvogel publication shows that differential centrifugation and fractionation over a linear sucrose gradient taught in the instant specification can be used to isolate exosomes that comprise MHC I and/or MHC II molecules, and thus confirms that undue experimentation is not required. The Zitvogel article also confirms the operability of the specific parameters of differential centrifugation and fractionation in linear sucrose gradients taught in the instant specification for isolating antigen-presenting vesicles that comprise MHC I and/or MHC II molecules. Western blot and immunoprecipitation analyses to detect the presence of MHC I molecules as well as MHC II molecules can be carried out using techniques well known to those in the art at the time of filing of the application, and by following the procedures taught in the instant specification (page 3, line 1 through page 4, line 11; page 8, line 4 through page 10, line 9; and page 12, line 31 through page 13, line 13 of the original specification), but using anti-MHC class I antibodies readily available to those in the art (please see Zitvogel *et al.*, cited by the Examiner, as well as Scott et al. (1995) *J. Immunol.* 155:143-148; Kaufman, *et al* (1995) *Proc. Natl. Acad. Sci.* 92:6484-

6488; Atta, *et al* (1995) *Clin. Exp. Immunol.* 101:121-126; Khilko, *et al* (1995) *J. Immunol. Methods* 183:77-94; Rangel, *et al* (1995) *Eur. Cytokine. Net* 6:195-202, all previously cited by Applicants). The work of Zitvogel, *et al* demonstrates the correctness of Applicants' assertion that "the exosomes possess MHC II and/or MHC I molecules at their surface" and that the detailed differential centrifugation and fractionation procedures taught in the instant specification apply to the isolation of antigen presenting vesicles that comprise MHC I and/or MHC II molecules.

3. Methods of using vesicles comprising MHC class I protein are provided, and are known to those skilled in the art.

With regard to the Examiner's statement that "guidance regarding the ...using of said exosomes is disclosed in the context of class II only," the specification, contrary to the Examiner's statement, does indeed teach how to use the claimed vesicles. Line 3 on page 7 of the specification refers to "the ... foremost use of these vesicles", in regard to the preceding lines comprising "MHC I or II" and vesicles "with desired peptides having the right binding motiv (sic) to fit in the respective MHC" (emphasis added). Uses for said vesicles include vaccines designed "to elicit an immune response against any proteinacious substance which has peptide antigens that can be presented in the context of MHC", and which may comprise "suitable adjuvants, if necessary, carriers, if necessary, ecxipients (sic) for administration, etc." (page 7, lines 6-13). Page 7, lines 14 -19 states "The vaccines can be used in the prophylaxis of many disorders, such as infections, immune disorders, malignancies, etc." "Very important applications will of course be the treatment or prophylaxis of AIDS, eliciting immuneresponses (sic) agains (sic) tumours and the like" (page 7, lines 14 - 19). Also, other important applications of the vesicles "is that they may be used to induce tolerance to certain antigens, for instance, by giving large doses of the vesicles orally" (page 7, lines 20-23). Applicants respectfully bring to the Examiner's attention that these annotations refer not only to MHC class II vesicles but also specifically to MHC class I vesicles (see page 6, line 3 through page 7, line 2. Zitvogel et al. have also demonstrated the operability of MHC class I bearing exosomes for stimulating T cells and for treatment of cancer.

The skilled artisan also knows how to use vesicles to stimulate T cells, and methods for the stimulation of T cells are well known. Furthermore, the present application demonstrates how to use antigen-presenting vesicles to stimulate T cells (see legend to Figure 4, page 10, lines 12-27 of the original specification) in the context of MHC class II vesicles, but the procedure is the same regardless of the antigen source, as is well known to those of skill in the art. Empirically adjusting for the number of cells that are used as the source of vesicles constitutes routine experimentation. These constitute reasons why "the presentation of peptides as antigens, for the stimulation of for instance T-cells" (page 7, lines 5-6) would be successful with vesicles prepared for said stimulation comprising "MHC I and II" (page 6, line 34) and "desired peptides having the right binding motiv (sic) to fit in the respective MHC" (page 7, lines 1-2).

4. One of skill in the art knows how to use vesicles as vaccines

One of skill in the art knows how to use vesicles as vaccines. As stated above, vesicles may be used to produce vaccines designed "to elicit an immune response against any proteinacious substance which has peptide antigens that can be presented in the context of MHC", and which may comprise "suitable adjuvants, if necessary, carriers, if necessary, ecxipients (sic) for administration, etc." (page 7, lines 6-13). Page 7, lines 14 -19 states "The vaccines can be used in the prophylaxis of many disorders, such as infections, immune disorders, malignancies, etc." "Very important applications will of course be the treatment or prophylaxis of AIDS, eliciting immuneresponses (sic) agains (sic) tumours and the like" (page 7, lines 14 - 19). Vesicles may also "be used to induce tolerance to certain antigens, for instance, by giving large doses of the vesicles orally" (page 7, lines 20-23). Applicants reiterate that these lines refer to vesicles comprising both MHC class I or MHC class II page 6, line 3 through page 7, line 2. Substituting MHC class I vesicles for any other antigen in a vaccination protocol at most constitutes routine experimentation.

5. The Zitvogel reference does not teach unpredictability of MHC class I expression on exosomes

Regarding the Examiner's statement that "in view of the lack of predictability concerning MHC class I expression on exosomes as demonstrated by the post-filing date Zitvogel reference", the fact that the authors had not previously observed of MHC class I expression on exosomes, and that "it was unexpected that multivescular late endosomes and exosomes in dendritic cells bear MHC I class molecules" (paper no. 16, page 3, lines 4-5) does not make the expression of MHC class I on exosomes unpredictable in an operative sense. "Unexpected" or "surprising" results must be reproducible in order to serve as a basis for a peer-reviewed journal article (such as the Zitvogel article). Zitvogel teaches that human monocyte-derived dendritic cells secrete exosomes that express both MHC class I and class II. Applicants note that the thrust of the Zitvogel reference is the use of exosomes as "a novel cell-free therapeutic cancer vaccine" made possible because "endosomes and exosomes produced by dendritic cells bear MHC class I and II molecules. Tumor peptide-loaded dendritic cells (DC)-derived exosomes "induced CTL priming *in vivo* and suppressed growth or induced complete regression of several established murine tumors" (page 594, column 2, lines 5-10). Zitvogel also states "Co-localization of MHC class I and class II molecules in DCs' endosomes was also seen in confocal microscopy" (page 595, column 1, lines 12-14), "DCs secrete a population of endosome-derived membrane vesicles which can bear both MHC I and II molecules" (page 595, column 1, lines 19-21), "(murine) Bone marrow derived-DCs cultured in IL-4 and GMCSF were analyzed by confocal and electron microscopy and found to contain multivesicular late endosomes bearing both MHC I and MHC II molecules" (page 595, column 1, lines 36-41) and "These DCs expressed low levels of B7.2, CD40, MHC class I and II molecules, as expressed by flow cytometry analysis. Upon LPS stimulation, the expression of these molecules was upregulated. The markers expressed by the BM-DC derived-exosomes were characterized and quantified by electron microscopy...and western blotting" (page 595, column 1, lines 46-51), Thus, the Zitvogel publication confirms the observation that MHC class I proteins may be found in the external membrane of endosomes, in intraluminal vesicles, at the cell surface, and in exosomes, under different circumstances. Perhaps most significantly, Zitvogel et al. provide methods for culturing bone-marrow derived-DCs to express MHC class I proteins, these markers may manipulated by upregulating

their expression, and MHC class I proteins may be characterized AND quantified using standard, published methods. Applicants respectfully submit that these statements in the Zitvogel reference confirm the predictability, rather than unpredictability, of MHC-I expression on exosomes.

6. The Zitvogel reference demonstrates operability of MHC class I vesicles in the context of the subject invention

Contrary to the Examiner's assertion, the Zitvogel reference demonstrates operability of MHC class I vesicles in the context of the subject invention: i.e. vesicles for disclosed uses comprising MHC class I.

(a) *Stimulation of T cells*. "To assess the capacity of DC-derived exosomes to induce effective T cell-mediated immune responses *in vivo*", the Zitvogel article "evaluated their antitumor effects in tumor-bearing mice"(page 595, column 1, lines 33-35). These studies demonstrated feasibility for triggering "tumor specific T cell responses (most likely involving CD8+ CTLs) through the secretion of antigen presenting vesicles" comprising MHC class I and MHC class II proteins (page 594, column 2, lines 3-5). Exosomes bearing MHC class I and class II proteins "directly primed tumor specific CTL responses in tumor-bearing mice ... a single injection of exosomes derived from DCs pulsed with the relevant peptides efficiently primes specific antitumor CTL responses *in vivo*" (page 596, column 2, lines 12-27).

(b) *Inducing tumor regression*. Several experiments were performed by Zitvogel et al. to demonstrate that exosomes bearing MHC class I molecules display antitumor activity (page 595, column 2, line 7 through page 596, column 2, line 27). Exosomes were produced from bone marrow-derived dendritic cells cultured in IL-4 and GMCSF, known to induce expression of both class I and class II molecules (see above discussion (5)). Tumor peptide-loaded DC-derived exosomes "induced CTL priming *in vivo* and suppressed growth or induced complete regression of several established murine tumors" (page 594, column 2, lines 5-10), and "tumor growth stopped in mice treated with exosomes derived from autologous tumor peptide pulsed DCs, and 40-60 of mice were tumor-free at day 60" (page 596, column 1, lines 8-10).

In view of the foregoing, Applicants respectfully assert that the instant specification and the state of knowledge present at the time the subject application was filed, properly enables those of ordinary skill in the art to make and to use an antigen-presenting vesicle comprising an MHC I molecule. Accordingly, the Examiner is respectfully requested to withdraw the rejection of Claims 3-4, 6 and 13 under 35 USC §112, first paragraph.

CONCLUSION

No new matter has been introduced by these amendments and no new search is required. In view of these amendments, it is submitted that this application is now ready for allowance. Early notice to that effect is solicited. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 328-4400.

Respectfully submitted,

Dated: July 17, 2001

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PATENT

ATTORNEY DOCKET NO. VEOC.001.01US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Geuze and Melief	)	Examiner: Amy DeCloux
Serial No.: 09/011,167	)	Art Unit: 1644
Filed: October 5, 1998	)	**Marked-up Claims**
For: **CELL DERIVED ANTIGEN PRESENTING VESICLES**	)	

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

These marked-up claims accompany the attached Response to the Advisory Action mailed May 4, 2001, for the above-identified patent application and is submitted by the extended due date of July 17, 2001.

AMENDMENTS

IN THE CLAIMS:

Cancel Claim 2.

9. (Three Times Amended) [A method for obtaining antigen presenting vesicles] An antigen presenting vesicle having a membrane and a major histocompatibility complex (MHC) class I protein, [said method comprising] obtained by the step of:

recovering a membrane-enriched fraction obtained by differential centrifugation of membrane-containing fractions of cell culture supernatants or lysates of antigen presenting cells [whereby fractions containing said antigen presenting vesicles are obtained].

CERTIFICATE OF FIRST CLASS MAILING

I hereby certify that this paper or fee is being deposited with the United States Postal Service as first class mail in an envelope addressed to BOX AF, Assistant Commissioner of Patents and Trademarks, Washington, D.C. 20231
on July 17, 2001.

Signature: _____

Printed Name: Jeffrey M. Libby

Cancel Claim 10.

11. (Amended) [A method for obtaining antigen presenting vesicles]An antigen presenting vesicle having a membrane and a major histocompatibility complex [(MHC) protein derived from MHC] class I[I] molecule, [said method comprising] obtained by the step of:

recovering a 70,000 x g pellet obtained by differential centrifugation of membrane-containing fractions of cell culture media or lysates of antigen presenting cells containing MHC class I[I, whereby fractions containing said antigen presenting vesicles are obtained].

12. (Amended) A [method for obtaining] purified antigen presenting vesicle[s] having a membrane and a major histocompatibility complex [(MHC) protein derived from MHC] class I[I] molecule, [said method comprising] obtained by the step of:

recovering a fraction having a buoyant density of 1.10 to 1.22 g/ml from a 70,000 x g pellet obtained by differential centrifugation of membrane-containing fractions of cell culture supernatants or lysates of antigen presenting cells containing MHC class I[I, whereby purified antigen presenting vesicles are obtained].

13. (Twice Amended) An antigen presenting vesicle free from its natural surroundings, comprising:

a membrane and a major histocompatibility complex (MHC) class I protein or a functional derivative or fragment thereof, wherein said antigen presenting vesicle is obtainable from [a] an antigen presenting cell.

Respectfully submitted,

Dated: July 17, 2001

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